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# The effect of hormone replacement therapy on endothelial function in postmenopausal women with hypertension

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**Background:**

## Summary

Endothelial dysfunction has been implicated in the pathophysiology of cardiovascular diseases, including arterial hypertension. The present study was undertaken to assess endothelial function in postmenopausal women with arterial hypertension receiving hormone replacement therapy and antihypertensive treatment.

**Material/Methods:**

A group of 76 women with natural menopause and essential mild to moderate arterial hypertension entered the study. Forty women received a transdermal, combined hormone replacement therapy (HRT) of 17 $\beta$ -estradiol and norethisterone acetate, whereas 36 served as controls. At baseline and at 3 and 12 months, all patients underwent 24-hr blood pressure monitoring and an exercise test, before which, at peak exercise, and after a 15-min recovery period venous blood was drawn to measure the level of nitrite/nitrate (NOx) according to the Griess method.

**Results:**

At 3 and 12 months after beginning HRT, the level of NOx at rest was slightly increased, with marked individual differences in response to HRT. In women not receiving HRT, NOx did not change. In the HRT group, 52.5% at 3 months and 47.5% at 12 months had significantly increased levels compared with the baseline values ( $17.8 \pm 6.7$  vs.  $32.8 \pm 4.5$  vs.  $28.7 \pm 1.1$   $\mu$ mol/l;  $p=0.002$ ). The increased NOx level in responders was associated with decreased LDL cholesterol ( $3.62 \pm 1.2$  vs.  $3.53 \pm 1.3$  vs.  $2.6 \pm 0.6$  mmol/l;  $p=0.01$ ). At 12 months, blood pressure values did not differ from those at baseline in either group.

**Conclusions:**

The significant increase of NOx in half of the women receiving HRT suggests that only responders experience the cardioprotective effects of HRT.

**key words:**

hormone replacement therapy • endothelium • hypertension

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## BACKGROUND

Arterial hypertension is one of the major coronary risk factors for increasing morbidity and mortality from cardiovascular diseases and stroke. While most studies of hypertension have focused on men, women also experience significant hypertension-related morbidity and mortality. However, the incidence of hypertension and cardiovascular disease is significantly lower in women than in men until the onset of menopause, at which time cardiovascular incidence increases dramatically in women and eventually approaches that of men [1]. These observations indicate that estrogen loss contributes to the menopause-related increase in blood pressure and cardiovascular disease and suggest that the use of hormone replacement therapy could decrease cardiovascular disease in postmenopausal women. However, new findings from the Women's Health Initiative study [2] suggest that estrogen therapy has few positive benefits and some significant negative effects on the health of postmenopausal women. Conversely, some clinical and basic research studies indicate that estrogen replacement therapy beneficially reduces blood pressure [3]. Estrogens have been suggested to exert vasoprotective effects as they influence the renin angiotensin system, have antioxidant properties, and may act as calcium-blocking agents [4,5]. Thus, HRT would be expected to leave blood pressure unchanged or to actually promote a blood pressure reduction in postmenopausal hypertensive and normotensive women. Nevertheless, the use of hormone replacement therapy in hypertensive women remains a controversial issue. Caution is recommended, as the effect of the therapy on blood pressure is equivocal.

Endothelial dysfunction has been implicated in the pathophysiology of cardiovascular diseases, including arterial hypertension, atherosclerosis, hypercholesterolemia, coronary artery disease, and heart failure. Endothelial function has been shown to deteriorate with age in both sexes. However, the accumulating data indicate that age-related changes in women are more pronounced with loss of ovarian function, which strongly suggests a relationship with sex hormones and their influence on vascular endothelial function. This has been confirmed by experimental [6] and clinical studies demonstrating abnormal vasodilatation in women after surgical and natural menopause [4–8], especially in the presence of atherosclerotic risk factors [9].

Endothelial dysfunction is an imbalance among endothelium-derived factors in favor of vasoconstrictive, pro-atherogenic substances over vasodilative ones [10]. Nitric oxide is an important anti-atherogenic factor, and reduced nitric oxide availability has been suggested to serve as a hallmark of endothelium dysfunction. In experimental studies, a strong relation between hypertension and endothelial cell morphological and functional alteration has been confirmed [10]. In postmenopausal women receiving estrogen replacement therapy (ERT), nitric oxide returns to a level comparable to its physiological level in women before menopause [11]. This is associated with improved endothelial

function, which may be immediate [12] or after long-term treatment [13,14]. The available experimental and clinical studies on the effect of estrogen replacement therapy on the metabolism of nitric oxide are limited to postmenopausal women with normal blood pressure.

Taking into account the complex relations between endothelial function, menopause, and hypertension, we focused on the role of endothelium dysfunction in hypertensive postmenopausal women and its possible restoration through the use of hormone replacement therapy combined with antihypertensive treatment. The present study was undertaken to assess the influence of combined transdermal hormone replacement therapy on blood pressure and endothelial function in postmenopausal women with arterial hypertension receiving antihypertensive treatment.

## MATERIAL AND METHODS

The study was designed for women treated at the Outpatient Unit of the 1<sup>st</sup> Cardiac Department, Jagiellonian University Medical College. A group of 76 women (mean age:  $52.5 \pm 5.8$  years) with natural menopause and essential mild to moderate arterial hypertension with a duration of  $5.9 \pm 5.0$  years entered the study. The mean time of amenorrhea was  $54.2 \pm 47.4$  months, FSH was  $>21$  U/l (mean:  $74.6 \pm 27.1$ ), and estradiol  $<50$  pg/ml (mean  $17.3 \pm 9.5$ ). Subjects with coronary artery disease, heart failure, diabetes, obesity, coagulation disorders, liver damage, hyperlipidemia, or imminent neoplastic process were excluded from the study. None of the patients had received hormone replacement therapy before. Hypotensive treatment consisted of beta blockers, calcium antagonists, diuretics, and angiotensin-converting enzyme inhibitors. The type and dosages of the antihypertensive agents remained unaltered during the 12-month follow-up. None of the patients received lipid-lowering treatment.

After gynecological examination, women with a low level of hormones and/or severe postmenopausal symptoms were offered transdermal hormone substitution with  $17\beta$ -estradiol and norethisterone acetate (Estracomb TTS 50, Novartis). The forty women who accepted the treatment received hormone replacement therapy, whereas the 36 women who chose not to take HRT did not receive the treatment. All patients underwent cytology of the vaginal epithelium as well as ultrasound examination, mammography and densitometry. The enrolled women had the basic measurements taken in a clinical setting: sedimentation rate, blood morphology, general urinary test, proteins, creatinine, total bilirubin, transaminase, serum alkaline phosphatase, and coagulation parameters (INR, APTT). Each patient underwent a physical examination.

The study protocol was approved by the Jagiellonian University Ethics Committee. The enrolled patients were informed about the aim of the study and gave their written consent.

**Table 1.** Characteristics of study groups: Group 1 – hypertensive women receiving hormone replacement therapy, Group 2 - hypertensive women without hormone replacement therapy.

	HRT Group 1	Without HRT Group 2	p
Age [years]	52.8±5.3	53.6±5.9	NS
BMI [kg/m <sup>2</sup> ]	28.3±4.8	27.0±3.6	NS
WHR	0.87±0.06	0.86±0.08	NS
Duration of hypertension [years]	5.8±4.1	6.6±4.5	NS
SBP [mmHg]	143.1±19.9	141.8±16.8	NS
DBP [mmHg]	89.5±6.4	90.6±7.9	NS
24 h SBP/DBP [mmHg]	128.7±16.3 / 85.3±8.0	130.3±12.7 / 84.7±9.1	NS
Day-time SBP/DBP [mmHg]	133.4±17.4 / 89.5±8.4	133.8±12.6 / 88.4±8.3	NS
Night-time SBP/DBP [mmHg]	119.4±16.2 / 77.1±9.7	123.5±14.6/77.5±10.9	NS
Total cholesterol [mmol/l]	6.1±1.2	6.4±1.1	NS
LDL cholesterol [mmol/l]	3.9±1.0	4.0±1.1	NS
HDL cholesterol [mmol/l]	1.5±0.4	1.5±0.3	NS
TG [mmol/l]	1.8±0.7	2.0±0.7	NS

HRT – hormone replacement therapy, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, NS – not significant

### 24-hr non-invasive ambulatory blood-pressure monitoring (ABPM)

At baseline and at 3 and 12 months, all patients underwent 24-hr ABPM using a SpaceLabs 90207 oscillometric recorder (SpaceLabs Inc, Redmond, Washington, USA) with recordings every 20 min during the day (6.00–22.00) and every 30 min at night (22.00–6.00). Day and night time intervals were defined arbitrarily for all patients because of the identical hospital regime. The recorder was mounted between 8.00 and 9.00 a.m.

We measured systolic and diastolic blood pressure separately over the entire 24 hours and at night the nocturnal systolic and diastolic blood pressure drop. We analyzed blood pressure variability from the standard deviations of all the blood pressure measurements taken over the entire 24 hours.

### Biochemical tests

At baseline and at 3 and 12 months all patients underwent an exercise stress test according to Bruce's protocol. Before, at peak exercise, and after a 15-min recovery period, venous blood was drawn to measure the level of nitrite/nitrate (NO<sub>x</sub>). The level of endogenous nitric oxide was determined from the measurement of nitrite/nitrate (NO<sub>x</sub>) concentration in venous blood with the modified Griess method using NADPH reductase [15].

At baseline and at 3 and 12 months we measured **total cholesterol, HDL and LDL cholesterol, and triglycerides**. Total cholesterol and triglycerides were measured with the enzymatic-colorimetric method using CHOD-PAP Boehringer Mannheim kits on RA 1000. HDL cholesterol was measured after precipitation of lipid fractions with heparin and MN2+. LDL cholesterol was calculated according to the Friedewald formula.

**FSH and estradiol** were measured using the MEIA kits of Abbott (sensitivity for estradiol 1 ng/ml, for FSH 0.5

**Table 2.** Antihypertensive treatment in the two studied groups: Group 1: hypertensive women receiving HRT and Group 2: hypertensive women not receiving hormone replacement therapy.

	HRT Group 1	Without HRT Group 2
Regimen		
Single drug therapy, %	53	42
Two antihypertensive drugs, %	41	53
Three antihypertensive drugs, %	3	5
Four antihypertensive drugs, %	3	0
Antihypertensive Drug Used (monotherapy or in combination)		
ACE Inhibitor %	53.3	46.6
Calcium antagonist %	10.0	3.3
Beta-blocker %	60.0	73.3
Thiazide diuretic %	43.3	26.6
Others %	2.7	3.3

mIU/ml) at the same time intervals as the remaining biochemical parameters.

The biochemical tests were performed at the Department of Clinical Biochemistry, Chair of Clinical Biochemistry and Diagnosis, Jagiellonian University Medical College.

### Statistical analysis

Statistical analysis was done using Statistica 5.0 PL for Windows. Basic statistics such as means, standard deviations, and the normal distribution were analyzed. Inter-group differences were tested using the chi-square test, Student's t-test and one-way variance analysis. Only two-sided tests were used. A  $p < 0.5$  was considered as statistically significant.

### RESULTS

Table 1 summarizes the anthropometric parameters and blood pressure values in the study population and

**Table 3.** Changes from baseline of 24-hr ambulatory blood pressures in the group receiving hormone replacement therapy (HRT) and without HRT – 3 and 12 months of follow-up (means (95 % CI)) and 95 % CI for differences between groups.

	HRT Group 1	Without HRT Group 2	95 % CI for differences between groups
<b>Three months</b>			
Δ 24-h SBP [mmHg]	-2.5 (-13.8 to 8.8)	-1.2 (-5.0 to 2.6)	(-12.0 to 9.4)
Δ 24-h DBP	-3.2 (9.4 to 2.8)	-1.1 (-3.6 to 1.3)	(-8.1 to 3.9)
Δ day-time SBP [mmHg]	-5.2 (-17.8 to 7.4)	-1.8 (-6.3 to 2.6)	(-15.5 to 8.7)
Δ day-time DBP	-4.7 (-10.8 to 1.5)	-1.4 (-4.2 to 1.3)	(-9.5 to 2.9)
Δ night-time SBP [mmHg]	1.4 (-11.8 to 14.7)	-1.5 (-5.8 to 2.8)	(-9.7 to 15.5)
Δ night-time DBP	-1.4 (-9.4 to 6.7)	-0.8 (-3.6 to 2.0)	(-8.3 to 7.1)
<b>Twelve months</b>			
Δ 24-h SBP [mmHg]	0.3 (-7.1 to 7.8)	-0.3 (-5.1 to 4.4)	(-8.2 to 8.2)
Δ 24-h DBP	-0.7 (-5.9 to 4.4)	0.1 (-3.1 to 3.3)	(-6.26 to 5.06)
Δ day-time SBP [mmHg]	-1.0 (-9.2 to 7.2)	-0.8 (-6.1 to 4.4)	(-9.2 to 8.8)
Δ day-time DBP	-1.2 (-6.8 to 4.4)	-0.4 (-3.9 to 3.0)	(-6.82 to 5.22)
Δ night-time SBP [mmHg]	2.7 (-4.1 to 9.6)	0.8 (-3.7 to 5.3)	(-5.79 to 9.59)
Δ night-time DBP	-0.2 (-5.1 to 4.4)	1.1 (-2.1 to 4.2)	(-6.62 to 4.02)

**Table 4.** Nitrite/nitrate (NOx) and LDL cholesterol levels at baseline and at 3 and 12 months in women receiving hormone replacement therapy, divided into responders and non-responders.

	At baseline	At 3 months	At 12 months	p
<b>Responders n=21</b>				
NOx [ $\mu$ mol/l]	17.8±6.7	32.8±14.5	28.7±15.5	0.002
LDL [mmol/l]	3.62±1.2	3.3±1.3	2.6±0.6	0.01
<b>Non-responders n=19</b>				
NOx [ $\mu$ mol/l]	22.8±8.6	24.7±13.7	20.0±9.7	NS
LDL [mmol/l]	4.1±1.7	3.53±1.3	3.66±1.1	NS

**Table 5.** Estradiol and FSH levels at baseline and at 3 and 12 months in two groups: Group 1 – hypertensive women receiving hormone replacement therapy and Group 2 – hypertensive women without hormone replacement therapy.

	HRT	Without HRT	p
<b>At baseline</b>			
Estradiol [pg/ml]	19.8±9.9	18.4±8.6	NS
FSH [U/l]	72.6±28.7	76.0±30.4	NS
<b>At 3 months</b>			
Estradiol [pg/ml]	92.5±26.6*	18.9±9.2	P<0.01
FSH [U/l]	46.7±24.2*	70.6±32.8	P<0.05
<b>At 12 months</b>			
Estradiol [pg/ml]	174.4±94.8*	16.0±7.2	P<0.001
FSH [U/l]	20.5±8.9*	72.6±29.0	P<0.005

Table 2 contains data on the antihypertensive treatment. At baseline the study groups did not differ in age, BMI, waist/hip ratio, duration of hypertension, and blood pressure (Table 1).

### Blood pressure

At 3 months, women receiving hormone replacement therapy showed a tendency towards lower systolic and diastolic blood pressure. At 12 months, blood pressure values did not differ from those at baseline in either group (Table 3). In women without HRT, blood pressure remained unaltered.

### Endothelial function parameters

At 3 and 12 months after hormone replacement therapy, the level of nitrite/nitrate at rest was slightly increased, with marked individual differences in response to HRT (23.6±11.7 vs. 28.7±19.9 vs. 26.2±17.4  $\mu$ mol/l, NS). In women not receiving HRT, nitrite/nitrate did not change significantly during the 12 months of follow-up (24.8±11.7 vs. 25.1±14.7 vs. 21.6±13.2  $\mu$ mol/l, NS).

In the HRT group, 21 women (52.5%) at 3 months and 19 (47.5%) at 12 months had significantly increased levels of NOx compared with the baseline values (Table 4). The increased NOx level in responders was associated with decreased LDL cholesterol. The level of circulating estradiol was similar in women with and without increased NOx (216±56 vs. 207±64 pg/ml; NS) (Table 5).

### DISCUSSION

In the present study, postmenopausal women with mild to moderate arterial hypertension receiving antihypertensive treatment and HRT showed a tendency towards lower blood pressure at 3 months and a return to baseline values at 12 months. These findings are in accordance with the results of Lip et al. [16], who, in a prospective open study in women receiving antihypertensive treatment together with HRT, did not find significant differences in blood pressure. Similarly, the study of Sumino et al. [17] demonstrated that hormone replace-

ment therapy does not affect blood pressure in hypertensive postmenopausal women whose blood pressure has been well controlled prior to the initiation of HRT. Kornhauser et al. [18] conducted a randomized double-blind study over 90 days in menopausal women with mild to moderate hypertension. The 55 women first discontinued their antihypertensive medication. The patients were allocated to three groups: placebo, estradiol, and estradiol plus progesterone analogue. Blood pressure decreased in the placebo group and remained unchanged in the other two groups [18]. In contrast, Manhem et al. [19] tested the effects of transdermal estrogen on blood pressure in a placebo-controlled, double-blind, cross-over study which relied on 24-hr blood pressure monitoring. Transdermal estrogen had a small blood pressure-lowering effect on daytime blood pressure and did not interfere with nocturnal blood pressure [19]. However, larger trials with different primary end-points do not suggest that HRT influences blood pressure to a significant degree. The post-menopausal estrogen/progestin interventions (PEPI) trial focused on systolic blood pressure among various end-points [20]. Patients were randomized to placebo or conjugated estrogens with or without progestin, in a cyclic or consecutive fashion. No treatment-related effects on systolic blood pressure were observed. In the Heart and Estrogen/progestin Replacement Study (HERS), blood pressure was documented to be no different in the examined groups [21].

As blood pressure is determined by the interplay between peripheral vascular resistance and volume regulatory mechanisms, the effects of HRT on vascular and endothelial function should receive focused attention. It is generally recognized that arterial hypertension impairs endothelium-dependent vasodilation [22–24]. However, reports on the effect of essential hypertension on plasma nitrite/nitrate levels are controversial. Takayashi et al. [25] demonstrated that higher blood pressure is associated with higher nitrite/nitrate levels. Sagnella et al. [26] did not find any significant differences, whereas Node et al. [27] demonstrated lower nitrite/nitrate levels in hypertensives compared with controls. In the present study, at 3 and 12 months after transdermal combined hormone replacement therapy nitrite/nitrate levels were insignificantly increased with marked individual variations in response to HRT. It is noteworthy that only half of the women responded with an increase in nitrite/nitrate levels after HRT.

The present finding confirms the division of women receiving HRT into responders and nonresponders observed by Imthurn et al. [28] in a group of 26 normotensive postmenopausal women receiving oral combined hormone replacement therapy for 12 months. Similar to the present study, marked individual variations of  $\text{NO}_2/\text{NO}_3$  in response to estrogen replacement therapy were demonstrated by other investigators [28–30]. The individual capability to restore estrogen expression probably plays a role in this phenomenon [31]. Before menopause, estrogen receptors are constantly stimulated by estrogens, rapidly increasing nitric oxide in response to increased estrogens in the blood [32]. As estrogens up-regulate estrogen receptors [33],

the decreasing estrogens in women after menopause gradually down-regulate estrogen receptors in vessels, until their complete loss [31]. Women with endothelial injury, as in arterial hypertension, may be not capable of restoring estrogen receptors, and for this reason nitric oxide levels may rise only slightly or not at all in response to estrogens. The inability of nonresponders to restore estrogen receptors may also play a role in producing variable reactions to estrogen therapy [31].

Another reason for variations in NO response to hormone replacement therapy is decreased LDL cholesterol in responders, which inhibits nitric oxide synthase. In the present study, NOx increase in response to HRT was found only in those women in whom LDL cholesterol was significantly lower. Although nitric oxide increases mainly through estrogen receptors, the increased level of nitrites/nitrates in postmenopausal women may be partly due to decreased LDL cholesterol.

A negative effect of progesterone on estrogen-induced nitric oxide increase cannot be excluded, either. Progestagens have been found to blunt the cardioprotective properties of estrogens [14,34] and aggravate hypertension-related vascular injury [35]. Also in a study by Imthurn et al. [28], plasma nitrites/nitrates did not increase significantly when estrogens were combined with medroxyprogesterone or cyproterone acetate. These findings imply that progestagens, both natural and synthetic, irrespective of whether they are derivatives of testosterone or 17-OH progesterone, attenuate estrogen-induced increase in NO production. Progesterone probably inhibits estrogen-induced nitric oxide generation [36]. However, the mechanism by which progesterone decreases the estradiol-induced increase in NO production is not well understood.

In contrast, Nabulsi et al. [37] demonstrated that a combined HRT consisting of estrogens and medroxyprogesterone shows better cardioprotection even when compared with estrogens alone. Other investigators [28] also observed marked increases of NO metabolites in blood NOx at 6 months after combined HRT (transdermal estradiol plus norethisterone) between the first and 12<sup>th</sup> day of the menstrual cycle. They also found that a 12-month combined HRT (oral estradiol plus cyproterone acetate or medroxyprogesterone) results in a marked increase of NOx in blood, and the addition of progesterone does not attenuate the effect of estrogens [28]. Best et al. [38] also demonstrated a marked increase of NO metabolites in a small group of postmenopausal women receiving a combined HRT for 6 months. In their study, the level of NOx varied in response to estrogen therapy from subject to subject.

The marked increase of NO metabolites observed by the above-mentioned investigators compared with the increasing tendency in the present study may be accounted for by a different route of administration and type of hormone preparations. It should, however, be remembered that the studies quoted above were carried out in postmenopausal women with normal blood pressure, whereas our results were obtained in post-



menopausal women with mild to moderate arterial hypertension.

The question arises whether the varying NO response to HRT is related to varying estrogen levels in the blood, which in turn are associated with irregular HRT use. In order to exclude this possibility we measured estrogen levels at the same time points as the nitrites/nitrates.

In the present study, despite various NO responses to estrogens in responders and nonresponders, the level of circulating estradiol was similar in both groups. In contrast, Best et al. [38] demonstrated marked individual variations by the end of the study. Other investigators [28,36] did not measure the level of estradiol by the end of their studies. For this reason it cannot be excluded that the differences in the results obtained by other investigators and in the present study are related to varying levels of estrogens in the blood.

### Methodological limitations

In the present study, endogenous generation of nitric oxide was measured from the level of circulating nitrites/nitrates. The question arises whether this methodological approximation is appropriate. Nitric oxide is a labile molecule which degrades to nitrites and nitrates within several seconds after its formation. Studies *in vivo* have confirmed that endogenous changes in NO generation induced by endotoxins or L-methyl-nitroarginine may be assessed from changes in the circulating or excreted nitrites/nitrates [39–41]. Hibbs et al. [41], using L-guanidine-arginine as a substrate for nitric oxide synthesis, demonstrated that in humans increased nitrate formation in the blood occurs due to nitric oxide generated from labeled L-arginine. Although these studies suggest that nitric oxide is the primary source of circulating nitrites/nitrates, nitrates encountered in food should also be taken into account. However, it is rather improbable that changes in the circulating nitrites/nitrates in the present studies should be ascribed to diet. Firstly, nitrates in food are excreted in the urine within 18 hours after their digestion [42]. In the present study the level of nitrites/nitrates was measured at 14 hours after the last meal; therefore, most nitrates had been eliminated by that time.

Node et al. [27] found that plasma NO<sub>x</sub> stabilizes 12 hours after the last meal. Secondly, because the patients were their own controls, it can be assumed that dietary habits were similar before and during HRT, thus eliminating the effect of food nitrates. Thirdly, if the endothelium is not the only site of NO generation, changes in NO<sub>2</sub>/NO<sub>3</sub> levels are not necessarily associated with impaired endothelial function [43,44]. However, the amount of nitric oxide released from blood platelets is about 20 times smaller than endothelium-derived NO [45], thus justifying the choice of methodology.

The inflammatory process has a potential effect on the level of NO<sub>x</sub>. In our study, standard markers of the inflammatory process or clinical symptoms of atheroscle-

rosis were not found; therefore, we may assume that variations of NO<sub>2</sub>/NO<sub>3</sub> reflect changes in NO generation.

A methodological limitation is also the antihypertensive treatment in our study. As it is still unknown whether hormone replacement therapy may modify the effects of antihypertensive treatment, most of all whether it does not attenuate the effects of hypotensive agents, and whether antihypertensive treatment may alter the benefits of HRT, the present study was designed to prospectively study 76 postmenopausal women with pharmacologically controlled arterial hypertension. The hypotensive treatment was similar in both groups, i.e. receiving and not receiving HRT, and it included all classes of antihypertensive drugs (beta blockers, calcium antagonists, diuretics, and ACE inhibitors). The doses of the drugs were also similar in both groups and they remained unaltered throughout the 12 months. We may then assume that the lack of need to increase the doses of antihypertensive agents shows that HRT does not aggravate arterial hypertension, which has not been indisputable in light of elevated blood pressure when using contraceptives.

There is no doubt that all the parameters analyzed in the present study, including endothelial function, vascular structure and function, and lipid and carbohydrate metabolism, are modifiable by antihypertensive treatment. However, if we take into account the fact that at baseline both groups were similar with respect to these parameters, and hypotensive treatment was the same throughout the follow-up, beneficial changes in the lipid profile are due to HRT, indicating that hormones significantly control lipid metabolism, which attenuates endothelial injury and inhibits atherosclerosis. This is reflected in the increased NO generation in the vascular walls of patients receiving hormone replacement therapy compared with those who did not receive HRT. Varying endothelial response with respect to NO formation also seems to be unrelated to hypotensive treatment, because the same pattern was observed in both responders and nonresponders.

The last possible limitation of this study is that it was not designed as a double-blind, randomized, placebo-based study. In addition, the number of subjects included in the study was relatively small. Therefore, we cannot exclude the possibility that there is a selection bias in the results. Hence, our findings require further support in larger, placebo-controlled studies.

To sum up, the combination of hormone replacement therapy and antihypertensive treatment does not attenuate, and even may improve, the beneficial effects of these agents.

### CONCLUSIONS

One-year combined transdermal hormone replacement therapy with estrogens and progestagens does not significantly affect blood pressure values and variability in postmenopausal women with arterial hypertension. The significant increase of NO<sub>x</sub> in half of the women receiving HRT suggests that only responders experience the cardioprotective effects of HRT.

## REFERENCES:

- Rexrode KM, Manson JE, Lee IM et al: Sex hormone levels and risk of cardiovascular events in postmenopausal women. *Circulation*, 2003; 108: 1688-93
- Rossouw JE, Anderson GL, Prentice RL et al, Working Group for the Women's Health Initiative Investigators: Risk and benefits of oestrogen plus progestin in healthy postmenopausal women: principle results from the Women's Health Initiative randomized controlled trial. *JAMA*, 2002; 288: 321-33
- Scuteri A, Bos AJ, Brant LJ et al: Hormone replacement therapy and longitudinal changes in blood pressure in postmenopausal women. *Ann Intern Med*, 2001; 135: 229-34
- Gilligan DM, Badar DM, Panza JA et al: Acute vascular effects of estrogen in postmenopausal women. *Circulation*, 1994; 90: 786-91
- Lieberman EH, Gerhard MD, Uehata A et al: Estrogen improves endothelium-dependent, flow mediated vasodilation in postmenopausal women. *Ann Intern Med*, 1994; 121: 936-41
- Laudański K, Cudnoch-Jędrzejewska A: Effects of ovariectomy on the regulation of cardiovascular functions in female Wistar rats. *Med Sci Monit*, 2001; 7: 1188-92
- Herrington DM, Braden GA, Williams JK, Morgan TM: Endothelial-dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. *Am J Cardiol*, 1994; 73: 951-52
- Reis SE, Gloth ST, Blumenthal RS et al: Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. *Circulation*, 1994; 89: 52-60
- Gilligan DM, Badar DM, Panza JA et al: Effects of estrogen replacement therapy on peripheral vasomotor function in postmenopausal women. *Am J Cardiol*, 1995; 75: 264-68
- Gryglewski R, Chlopicki S, Uracz W, Marcinkiewicz E: Significance of endothelial prostacyclin and nitric oxide in peripheral and pulmonary circulation. *Med Sci Monit*, 2001; 7: 1-16
- Cicinelli E, Ignarro LJ, Lograno M et al: Acute effects of transdermal estradiol administration on plasma levels of nitric oxide in postmenopausal women. *Fertil Steril*, 1997; 67: 63-66
- Binko J, Majewski H: 17  $\beta$ -estradiol reduces vasoconstriction in endothelium denuded rat aortas through inducible NOS. *Am J Physiol*, 1998; 274: H853-59
- Roque M, Heras M, Roig E et al: Short-term effects to transdermal estrogen replacement therapy on coronary vascular reactivity in postmenopausal women with angina pectoris and normal results on coronary angiograms. *J Am Coll Cardiol*, 1998; 31: 139-43
- Jokela H, Dastidar P, Rontu R et al: Effects of long-term estrogen replacement therapy versus combined hormone replacement therapy on nitric oxide-dependent vasomotor function. *J Clin Endocrinol Metab*, 2003; 88: 4348-54
- Guevara I, Iwanek J, Dembińska-Kieć A: Determination of nitrite/nitrate in human biological material by the sample Griess reaction. *Clin Chim Acta*, 1998; 274: 177-88
- Lip GY, Beevers M, Churchill D, Beevers DG: Hormone replacement therapy and blood pressure in hypertensive women. *J Hum Hypertens*, 1994; 8: 491-94
- Sumino H, Ichikawa S, Kumakura H et al: Effects of hormone replacement therapy on office and ambulatory blood pressure in Japanese hypertensive postmenopausal women. *Hypertens Res*, 2003; 26: 369-76
- Kornhauser C, Malacara JM, Garay ME, Perez-Luque EL: The effect of hormone replacement therapy on blood pressure and cardiovascular risk factors in menopausal women with moderate hypertension. *J Hum Hypertens*, 1997; 11: 405-11
- Manhem K, Ahlm M, Milsom I, Svensson A: Transdermal oestrogen reduces daytime blood pressure in hypertensive women. *J Hum Hypertens*, 1998; 12: 323-27
- The writing group for PEPI Trial: Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. *JAMA*, 1995; 273: 199-208
- Grady D, Herrington D, Bittner V et al: For the Heart and Estrogen/progestin Replacement Study (HERS) Research Group. Cardiovascular disease outcomes during 6.8 years of hormone therapy. Heart and Estrogen/progestin Replacement Study follow-up (HERS). *JAMA*, 2002; 288: 49-57
- Garcia CE, Kilcoyne CM, Cardillo C et al: Effect of copper-zinc superoxide dismutase on endothelium - dependent vasodilation in patients with essential hypertension. *Hypertension*, 1995; 26: 863-68
- Linder L, Kiowski W, Buhler FR, Luscher TF: Indirect evidence for release of endothelium-derived relaxing factor in the human forearm circulation *in vivo*. Blunted response in essential hypertension. *Circulation*, 1990; 81: 1762-67
- Panza JA, Casino PRC, Kilcoyne CM, Quyyumi AA: Impaired endothelium dependent vasodilation in patients with essential hypertension: evidence that the abnormality is not at the muscarinic receptor level. *J Am Coll Cardiol*, 1994; 23: 1610-16
- Takahashi H, Nakanishi T, Nishimura M et al: Measurements of serum levels of nitrate ions in men and women: implications of endothelium-derived relaxing factor in blood pressure regulation and atherosclerosis. *J Cardiovasc Pharmacol*, 1992; 20(suppl 12): S214-S216
- Sagnella GA, Markandu ND, Onipinla AK et al: Plasma and urinary nitrate in essential hypertension. *J Hum Hypertens*, 1997; 11: 587-88
- Node K, Kitakaze M, Yoshikawa H et al: Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. *Hypertension*, 1997; 30: 405-08
- Imthurn B, Rosselli M, Jaeger AW et al: Differential effects of hormone replacement therapy on endogenous nitric oxide levels in postmenopausal women substituted with 17  $\beta$ -estradiol valerate and cyproterone acetate or medroxyprogesterone acetate. *J Clin Endocrinol Metab*, 1997; 82: 388-94
- Best PJM, Berger PB, Miller VM, Lerman A: The effect of estrogen replacement therapy on plasma nitric oxide and endothelin-1 levels in postmenopausal women. *Ann Intern Med*, 1998; 128: 285-88
- Ramsay B, Johnson MR, Leone AM, Steer PJ: The effect of exogenous oestrogen on nitric oxide production in women: a placebo controlled crossover study. *Br J Obstet Gynaecol*, 1995; 102: 417-19
- Losordo DW, Kearney M, Kim EA et al: Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. *Circulation*, 1994; 89: 1501-10
- Stefano G, Peter D: Cell surface estrogen receptors coupled to cNOS mediate immune and vascular tissue regulation: therapeutic implications. *Med Sci Monit*, 2001; 7: 1066-74
- Rosser M, Chorch L, Howard E et al: Changes in rat uterine estrogen receptor messenger ribonucleic acid levels during estrogen and progesterone induced estrogen receptor depletion and subsequent replenishment. *Biol Reprod*, 1993; 48: 89-98
- Lobo RA: The role of progestins in hormone replacement therapy. *Am J Obstet Gynecol*, 1992; 166: 1997-2004
- Wolinsky H: Effects of estrogen and progesterone treatment on the response of the aorta of male rats to hypertension. Morphological and chemical studies. *Circ Res*, 1972; 30: 341-49
- Rosselli M, Imthurn B, Keller PJ et al: Circulating nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 17  $\beta$ -estradiol and norethisterone acetate: a two-year follow-up study. *Hypertension*, 1995; 25(4 Pt 2): 848-53
- Nabulsi AA, Folsom AR, White A et al: Association of hormone replacement therapy with various cardiovascular risk factors in postmenopausal women. *N Engl J Med*, 1993; 328: 1069-75
- Best PJM, Berger PB, Miller VM, Lerman A: The effect of estrogen replacement therapy on plasma nitric oxide and endothelin-1 levels in postmenopausal women. *Ann Intern Med*, 1998; 128: 285-88
- Anggard E: Nitric oxide: mediator, murderer and medicine. *Lancet*, 1994; 343: 1199-206
- Azuma H, Sato J, Hamasaki H et al: Accumulation of endogenous inhibitors for nitric oxide synthesis and decreased content of L-arginine in regenerated endothelial cells. *Br J Pharmacol*, 1995; 115: 1001-04
- Hibbs JP, Westenfelder C, Taintor R et al: Evidence for cytokine-inducible nitric oxide synthase from L-arginine in patients receiving interleukin-2 therapy. *J Clin Invest*, 1992; 89: 867-77
- Jungersten L, Edlund A, Petersson AS, Wennmalm A: Plasma nitrate as an index of nitric oxide formation in man: analyses of kinetics and confounding factors. *Clin Physiol*, 1996; 16: 369-79
- Dusting GJ: Nitric oxide in cardiovascular disorders. *J Vasc Res*, 1995; 32: 143-61
- Cadwgan TM, Benjamin N: Evidence of altered platelet NO synthesis in essential hypertension. *J Hypertens*, 1993; 11: 417-20
- Zhou Q, Hellermann GR, Solomonson LP: Nitric oxide release from resting human platelets. *Thromb Res*, 1995; 77: 87-96